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(54) Bi-specific antibodies

(57) Bi-specific antibody molecules having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, and fragments thereof retaining the binding affinity of the whole molecule, are of value in treating disease, particularly neoplastic, viral and parasitic diseases. The cytotoxic molecules or fragments are targeted against selected target cells through use *in vivo* in conjunction with antibody molecules or fragments thereof having a binding affinity for the target cells. Alternatively, the bi-specific antibody molecules or fragments may be combined *in vitro* with such antibody molecules or fragments having a binding affinity for target cells, to provide a bi-specific antibody conjugate or fragment thereof having the binding affinities of its two components which may be used *in vivo* in treating disease.

Processes for fusing hybridoma cells and for the selection of the resulting polydomas, which produce the bi-specific antibodies are disclosed.

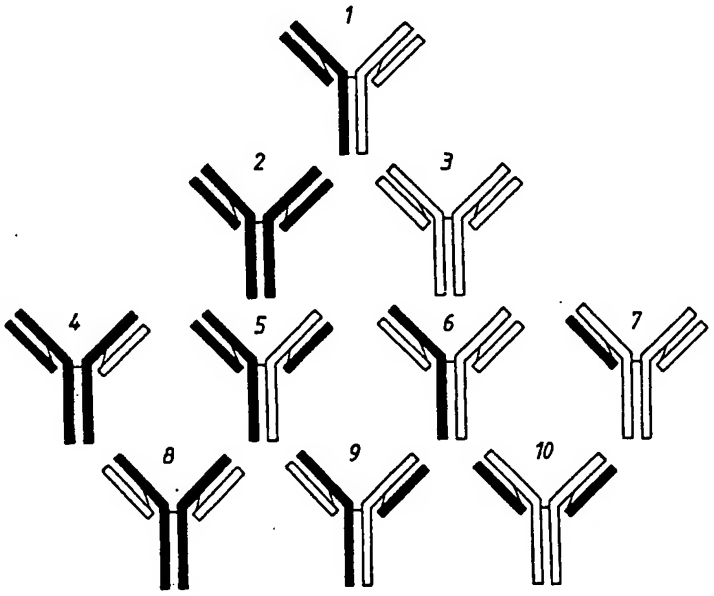

Fig.1

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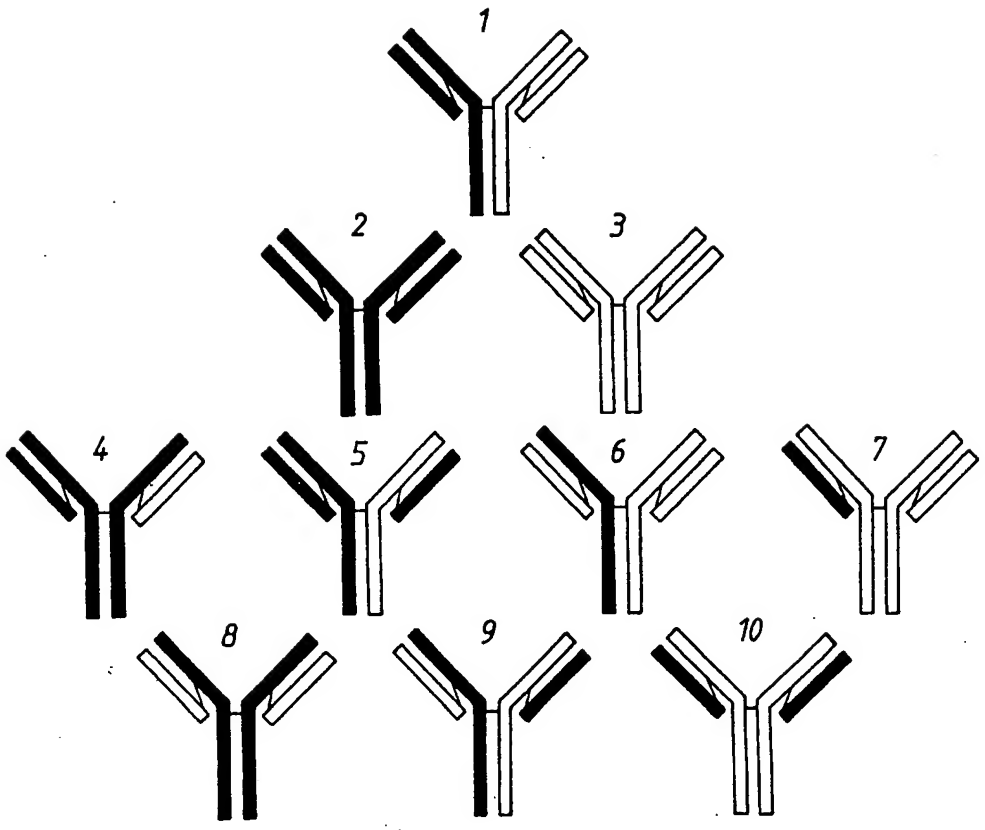


Fig. 1

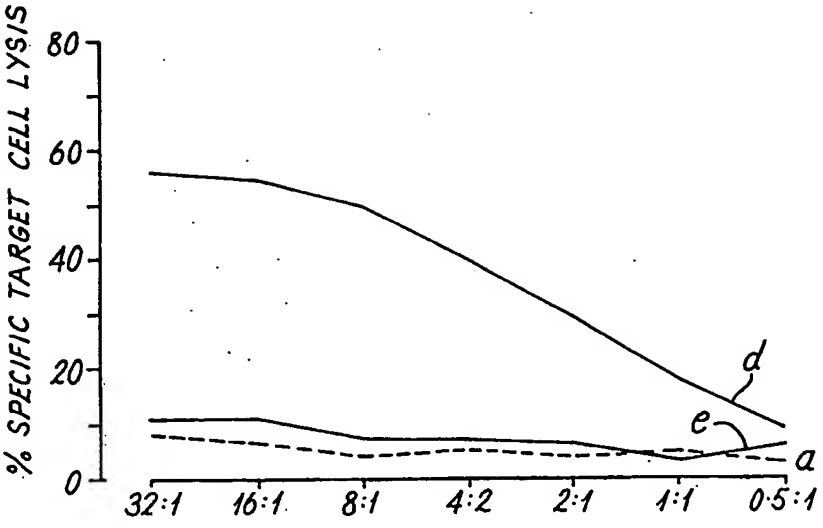
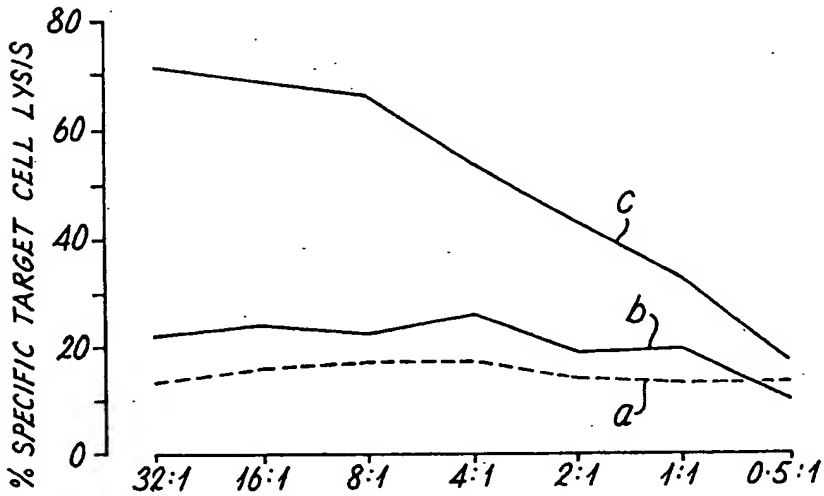

% SPECIFIC TARGET CELL LYSIS
80
60
40
20
0
d
e
a
32:1
16:1
8:1
4:2
2:1
1:1
0·5:1
EFFECTOR : TARGET RATIO

Fig. 2a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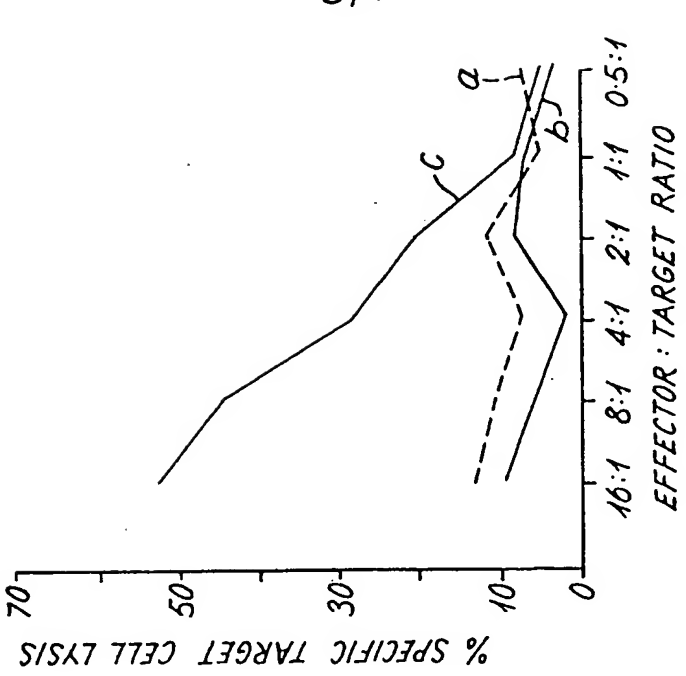
60
40
20
0
c
b
a
32:1
16:1
8:1
4:1
2:1
1:1
0·5:1
EFFECTOR : TARGET RATIO

Fig. 2b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50
30
10
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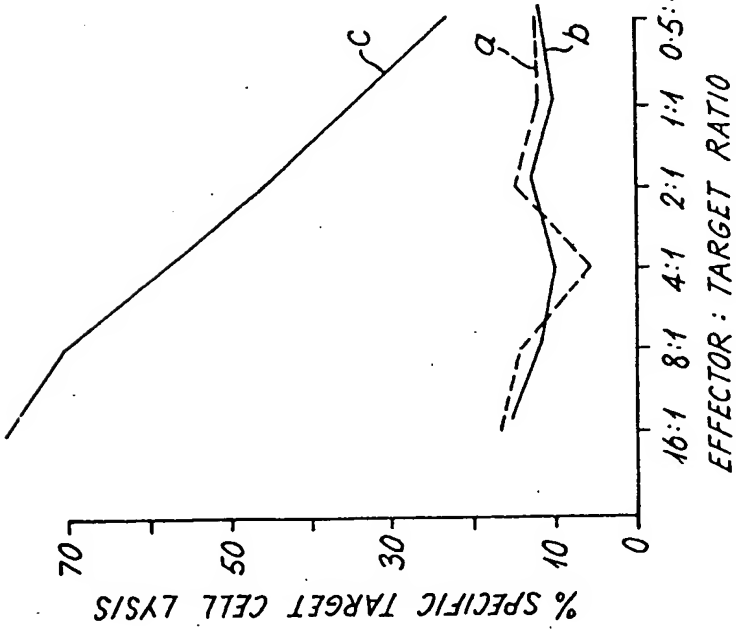
EFFECTOR : TARGET RATIO
a
b
c

Fig. 3b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50
30
10
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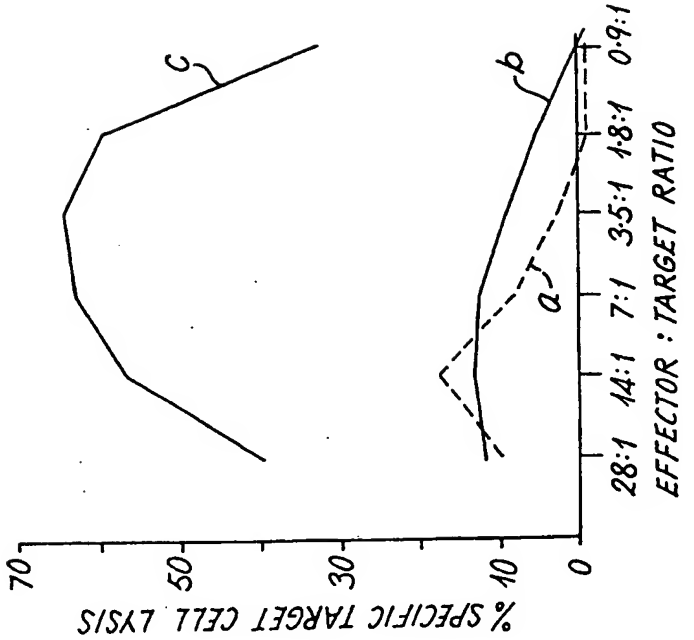
EFFECTOR : TARGET RATIO
a
b
c

Fig. 3a


% SPECIFIC TARGET CELL LYSIS
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50
30
10
0
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EFFECTOR : TARGET RATIO
a
b
c

Fig. 3d

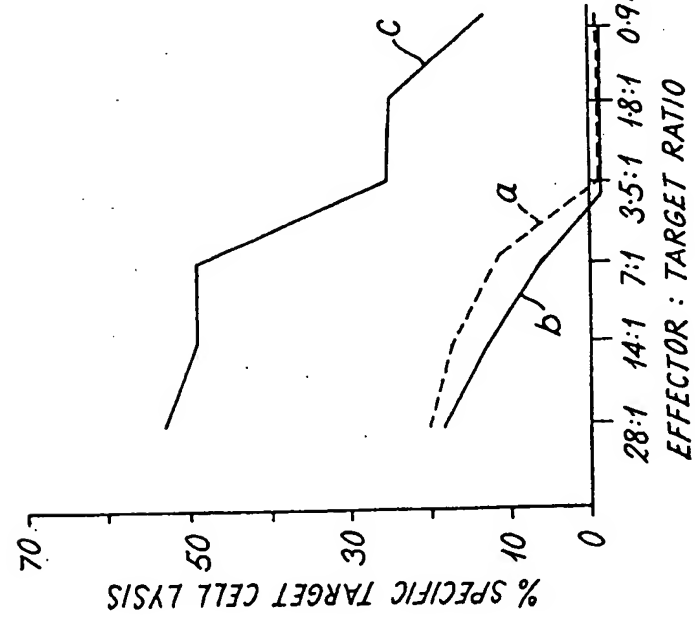
% SPECIFIC TARGET CELL LYSIS
70
50
30
10
0
28:1 14:1 7:1 3:5:1 1:8:1 0:9:1
EFFECTOR : TARGET RATIO
a
b
c

Fig 3c

SPECIFICATION

Antibodies

This invention relates to novel forms of antibody and their use as targeted cytotoxic agents, particularly in the treatment of neoplastic disease.

The treatment of neoplastic disease still remains an intractable problem despite the fact that a very wide range of cytotoxic agents has now been developed for use in the treatment of the disease. One recent approach utilises the phenomenon of effector cell retargeting (ECR) to destroy tumour cells. In this approach a bi-specific antibody is constructed which has both an anti-T cell and an anti-tumour antigen activity. In the first application of this technique (Staerz *et al*, Nature, 1985, *314*, 628, Perez *et al*, Nature, 1985, *316*, 354 and Liu *et al*, Proceedings of the National Academy of Sciences of the USA, 1985, 8648) a bivalent antibody conjugate was constructed by chemical means but in a further application of the technique (Staerz and Bevan, Proceedings of the National Academy of Sciences of the USA, 1986, *83*, 1453 and Immunology Today, 1986, *7*, 241) hybridoma technology has been employed to produce a bi-specific antibody molecule. The bi-specific antibody exerts its effect by binding both to a tumour cell or other form of target cell, such as a virally infected cell, and to a T-cell thereby effecting destruction of the former by the action of the latter.

The use of effector cell retargeting as described in the art does however have various inherent disadvantages. In particular, the bi-specific antibody will in general utilise to any significant extent only one of the natural cell killing mechanisms of which the body is capable in order to kill the tumour cells. It is an object of the present invention to provide an improved method for the destruction of tumour cells utilising the natural killing mechanisms of the body including the use of effector cell retargeting.

Accordingly the present invention comprises a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinity of the whole molecule.

It will be appreciated that the bi-specific antibody molecules of the present invention differ from those bi-specific antibodies of the 1985 Staerz *et al*, Perez *et al* and Liu *et al* papers in as far as, although being bi-specific, the present antibodies have the normal form of an antibody molecule in which two light chains and two heavy chains are present. By way of contrast the bi-specific antibodies described in 1985 were conjugates produced by the chemical cross linking of two normal antibody molecules and contained four light chains and four heavy chains. Although the bi-specific antibodies described in the 1986 Staerz and Bevan papers consist of antibody molecules rather than conjugates, the binding affinities of these molecules are of course different from those of the present invention and, moreover, the authors failed to appreciate the problems inherent in the use of their molecules as regards the killing of T-cells by the molecules. The present invention is further directed to the mitigation of this previously quite unrecognised problem. The 1985 Liu *et al* paper describes an antibody conjugate having one binding affinity for the human T3 complex and a second binding affinity for the idiotype of the surface immunoglobulin of a human B-lymphoma. Such an idiotype represents a true tumour specific transplanation antigen and is used to direct the antibody conjugate to tumour cells. The Liu *et al* antibody conjugate therefore essentially has anti-T-cell and anti-tumour cell affinities and, apart from its conjugate nature, may be distinguished from the bi-specific antibody molecules of the present invention which function against T-cells through binding with a further antibody having an anti-tumour cell, or other target cell affinity.

In their primary use the bi-specific antibody molecules described above are administered in conjunction with a separate antibody having a binding affinity for target cells. Such a procedure enables various of the body's cell killing mechanisms to be brought into play. Firstly, the antibody having a binding affinity for target cells can bind to a target cell and effect killing thereof through an Fc receptor mediated killing mechanism, for example via antibody-dependent cell-mediated cytotoxicity (ADCC) involving K cells, neutrophils and macrophages, via phagocytosis by macrophages and cells of the reticuloendothelial system, or via complement activation. Secondly, the resultant target cell/antibody system can bind further with the bi-specific antibody system through the immunoglobulin affinity of this system, the whole system then binding with a T-cell through the T-cell affinity of the bi-specific antibody system and utilising T-cell toxicity to destroy the target cell.

The bi-specific antibody molecules of the present invention function by binding to T-cells in order to direct their toxicity and the T-cell binding affinity of the antibody molecule may be specific for any T-cell receptor which will cause killing. Thus the receptor may be associated with the ability of the T-cells to kill either directly or indirectly through the assistance of other cells types or any other agent. Thus, the receptor may be one which is associated with the ability of cytotoxic T-cells to kill directly or with the ability of helper T-cells to assist killing by B-cells, or with any other indirect mode of killing (natural or artificial). Although receptors capable of activating direct

killing are of particular interest, it will be appreciated that many receptors ar associated with both direct and indirect modes of killing.

In particular, the binding affinity may be directed against one or both of the α and β chains which comprise the T-cell antigen specific receptor termed Ti, and which are present on the vast majority of T-cells, or it may be directed against the receptor-associated CD3 unit (previously identified as T3) as a whole or against one of the individual chains thereof. Thus, studies with human cells presently indicate that the T-cell receptor exists as a complex of two chains identified as α (M, about 50,000) and β (M, about 40.000) which are coded for by genes which are somatically rearranged in an analogous fashion to immunoglobulin genes. Each T-cell therefore possesses a unique rearrangement of genes coding for these two chains. These two chains are found in association with at least three other chains which comprise the CD3 complex and are identified as γ (M, about 25,000), δ (M, about 20,000) and ε (M, about 20,000).

Antibodies having a T-cell binding affinity, either for a known or a novel receptor, may be identified by an assay procedure which we have developed. Most hybridomas as well as secreting antibody have small amounts of cell surface antibody. Thus, for example, a mouse hybridoma making antibody against rat IgG2b is capable of trapping a rat IgG2b antibody by virtue of the small amount of antibody against rat IgG2b on its surface. If the hybridoma cells are labelled with a radioactive label, for example ^{51}Cr, and then incubated with a mixture of T-cells and monoclonal rat IgG2b antibodies against T-cells, the hybridoma cells will bind to the antibody which will in turn bind the T-cells, thereby leading to killing of the hybridoma and consequent release of the radioactivity. Such a procedure therefore provides a means of detectiny rat IgG2b antibodies with an appropriate T-cell binding capacity (i.e. those capable of inducing killing). By selection of an appropriate form of hybridoma such a screen can be conducted among antibodies of any species and class or subclass. The hybridomas producing antibodies detected in such an assay procedure may be used in preparing the bi-specific antibodies of the present invention by techniques described hereinafter.

Anti-non human T-cell/anti-immunoglobulin bi-specific antibody molecules are of interest, particularly in a research context, for example as a model system in the investigation of the important requirements for cell killing with animal tumours such as those of the rat and mouse. However, the major area of interest of the present invention is in human medicine and bi-specific antibodies having a first binding affinity directed against human T-cells are therefore of particular interest, these most usually being used in conjunction with a separate antibody having a binding affinity for target cells present in the human body. Using assay procedures such as that described above it is possible to select from among hybridomas producing monoclonal antibodies having the ability to bind with human T-cells those which are capable of triggering T-cell cytotoxicity, one example of such an antibody being the rat IgG2b antibody produced by the hybridoma YTH 12.5.14.2 (and all related subclones) which is described in Example 2 hereinafter. This antibody binds the T-cell antigen CD3 but other antibodies of different specificities may also be similarly selected. Other examples include the anti-human CD2 rat IgG2b antibodies produced by the hybridoma YTH 655(5)6 and YTH 616.7.10 (H.P. Tighe, Ph.D. Thesis entitled "Monoclonal antibodies against cell surface antigens involved in leukocyte function", University of Cambridge, 1987) and the anti-human CD3 mouse IgG2a antibodies produced by the hybridoma OKT3 which is described in Example 3, and also the group of anti-CD3 mouse Ig antibodies listed by Kurrle *et al*, Leukocyte Typing II, Volume 1, Human T-Lymphocytes, edited by Reinherz *et al*, Springer Verlag, 1985, page 137, and various of the antibodies listed for the Third International Workshop and Conference on Human Leukocyte Differentiation Antigens, Leukocyte Typing III, White Cell Differentiation Antigens, edited by McMichael, Oxford University Press, 1987, in particular those antibodies against the CD3 unit and also against the Ti receptor, as well as certain of the anti-CD2 antibodies. It will be appreciated that hybridomas producing all of these antibodies may of course be used in derivative forms (as reclones or subclones) or that analogous hybridomas of similar specificity may be employed.

The second binding affinity of the bi-specific antibody molecules according to the present invention may be directed against any of the variety of binding sites present in antibody immunoglobulins, being for example anti-allotype, anti-isotype (particularly -subclass), anti-species or to a lesser degree of preference, anti-idiotype, in its specificity. (The word isotype is used in the art to designate a particular class and/or subclass so that in the rat, for example, IgM, IgG1, IgG2a, IgG2b and IgG2c each constitute a separate isotype. In the present instance an anti-isotype binding affinity is most usually anti-subclass, for example anti-IgG1 etc. rather than anti-IgG, although it is possible for the affinity to be anti-class particularly when, as in the case of IgM, no subclasses exist.) In addition to such forms of binding affinity for immunoglobulins it will be appreciated that the immunoglobulin affinity of the bi-specific antibody molecule may also be directed against a site in the immunoglobulin exposed by the formation of a fragment thereof, for example the $F(ab')_2$ fragment of the anti-target cell antibody as described her -

inafter. Alt rnatively the affinity may be for an artificially creat d site on the immunoglobulin produced by the attachment of a hapten thereto. Such an affinity for immunoglobulin based upon anti-hapten binding may be utilised, for example, with fluorescein isothiocyanate labelled or biotinylated immunoglobulins.

Within the broad range of immunoglobulin specificities, however, there are certain preferences. Thus, the primary use of the bi-specific antibody molecules of the present invention is in indirect ECR where binding of the molecules to target cells is effected through a separate antibody molecule having a binding affinity for target cells. Accordingly, although it is possible for the immunoglobulin to which the bi-specific antibody molecules bind to be one which will result in direct ECR, it is preferred that the immunoglobulin is not one which causes the bi-specific antibody molecule to be targeted to cells, in particular tumour cells, without the use of a separate anti-target cell antibody. The immunoglobulin therefore preferably is not one which functions as a cell surface antigen, and particularly is not an antigen present on the surface of tumour cells. Less preferred types of immunoglobulin specifity are thus any specificity for an endogenously synthesised and expressed cell surface immunoglobulin, particularly one which appears on the surface of tumour cells. An anti-idiotype affinity is an especial case of a less preferred specificity, both as regards the functioning of many idiotypes as cell surface antigens and since an anti-idiotype can block antibody/-antigen binding. A specific example of a less preferred specificity involving a cell surface immunoglobulin and idiotype is the idiotype of the surface immunoglobulin of a human B-lymphoma, such as that described by Liu *et al, ibid,* which functions as a tumour specific cell surface antigen.

It will be appreciated, however, that for maximum freedom in selection of the anti-target cell antibodies which are employed in conjunction with the bi-specific antibodies, the immunoglobulin affinity of the latter is preferably as non-specific as possible. This preference is however subject to the proviso that the bi-specific antibody molecule is intended for use *in vivo* and that any undesirable side reaction with the patient's body should be avoided in as far as possible. In practice, the simplest way of avoiding undesired reactions between the bi-specific antibody molecule and immunoglobulin molecules naturally pr sent in the patient's body is for the immunoglobulin binding affinity to relate t a species other than that to which the T-cell receptor binding affinity r lates. Commonly the latter speci s is man. and the former may conveniently be another mammalian sp cies, for example the rabbit and particularly the mous or especially the rat. Thus, it is convenient if, for example, the bi-specific antibody will bind with any of th various classes of antibody as discussed h reinaft r and particularly with both IgM antibodies and IgG antibodies, including the various subclasses of the latter. Moreover, it is advantageous if the binding affinity is not totally species specific so that, for example, binding to at least both of rat and mouse antibodies is possible.

In practical terms, however, this may be too demanding a requirement and it may therefore be appropriate to use a small group of anti-immunoglobulin antibodies specific for the rat or mouse and possibly even for different subclasses thereof.

It should be appreciated that an important preference for the anti-immunoglobulin specificity is that it does not cause auto-reactivity. Thus, for example, if the bi-specific antibody molecule is itself composed of rat IgG2b heavy chains and rat kappa light chains, the anti-immunoglobulin specificity should exclude anti-rat IgG2b and anti-rat kappa. The bi-specific antibody molecule described in Example 2 hereinafter is a hybrid between the antibody YTH 12.5.14.2 (a rat IgG2b with a lambda light chain which is specific for the CD3 antigen) and the antibody RG11/15.5 (a mouse IgG2a with a kappa light chain which is specific for the rat kappa 1b allotype). This combination, which avoids auto-reactivity, can be used in conjunction with any rat antibody containing a rat kappa 1b allotype light chain.

As regards the anti-target cell antibodies used in conjunction with the bi-specific antibodies of the present invention, these may have a binding affinity for any antigen present on the surface of a target cell. The target cell may be any cell which may be beneficially removed from the body. Examples include virally infected cells (viruses themselves not normally being attacked by T-cells), the virus being of various types including the influenza and rabies viruses, and both parasitized cells and parasites themselves including those responsible for malaria, leprosy, trypanosomiasis and schistomiasis, as well as tapeworms and other parasitic worms such as helminths. The preferred target cells are however tumour cells, the anti-target cell antibodies having a binding affinity for any tumour-associated antigen. The ideal situation would be for the affinity to be for a tumour-specific antigen, i.e. an antigen found on tumour cells only and not on normal cells. However, the B-cell Ig idiotype which has been employed in the treatment of B-cell malignancies such as BCLL is on of th very few exampl s of such antigens which exist and in practic the tumour-associated antigen will also exist on normal cells. Preferably, the antig n is anomalously xpressed at higher levels or in an appropriate way on tumour cells thereby allowing an nhanced level of antibody-antigen reaction with the tumour cells but even this is not complet ly necessary. Thus, for xample even in the extrem case

where there is no differentiation between tumour cells and normal haemopoietic cells, the toxic effects of the treatment on the normal cells can be countered by the use of marrow transplants or of removal of the patient's own bone marrow prior to the treatment and its return thereafter, conveniently following separate treatment of the bone marrow *in vitro* for the removal of tumour cells. Examples of anti-tumour antibodies of particular interest are antibodies against antigens which define clusters of differentiation (CD) of the haemopoietic system recognised by groups of monoclonal antibodies standardised and characterised by International Workshops on Human Leukocyte Differentiation Antigens (Paris 1980, Boston 1983, Oxford 1986), and antibodies to the common acute lymphoblastic leukaemia-associated antigen CALLA, the carcinoembryonic antigen as expressed on human colon carcinoma, and the human melanoma-associated ganglioside GD3. A specific example is the human B-cell differentiation antigen CD19 which is expressed on normal B-cells and many malignant B-cells and B-cell lines.

The anti-target cell antibodies may be produced by classical techniques as a polyclonal preparation but are more conveniently produced as monoclonal antibodies using hybridoma technology, for example as described in US Patent 4,172,124 and in a wide range of scientific papers present in the literature.

The bi-specific antibody molecules described herein may be produced by the chemical linkage of the halves of two antibodies, which may be produced by classical techniques or by hybridoma technology, one of the first binding activity and another of the second binding activity. However, particularly preferred bi-specific antibody molecules according to the present invention are those produced directly by the techniques of hybridoma technology modified as necessary for the production of bi- rather than mono-specific antibodies. Such techniques for the preparation of bi-specific antibodies, in general, are described in European Patent Application 0068763 and PCT Application WO 83/03679 which relate broadly to bi-specific antibody molecules produced by hybridoma technology. Examples of myeloma starting materials for use both in the preparation of the bi-specific antibodies and also the antitarget cell antibodies are the Y3-Ag 1.2.3 myeloma of European Patent No. 001459 (C.N.C.M. No. I-078), the YB2/3.0.-Ag.20 myeloma of European Patent No. 0043718, the myeloma P3-X63-Ag8 (A.T.C.C. No. CRL 1597), the myeloma NS1/1-Ag4-1 (A.T.C.C. No. TIB 18) and the myeloma P3 (Staerz *et al*, Journal of Immunology, 1985, *134*, 3994–4000), as well as the various human myelomas referred to in the literature such as those of European Patent Applications 0062409 and 0148644, UK Patent 2086937 and US Patent 4529694. It is preferred that each hybridoma is either derived from a myeloma which does not express a light chain or is a myeloma light chain loss variant, i.e. being HL rather than HLK. The techniques used in the preparation of the bi-specific antibodies of the present invention closely parallel those described in U.K. Patent Application 2,144,147A which may also be applied in the case of the present invention, although the binding affinities of the fusion partners are of course different. We have, however, developed a variant of these techniques which has proved to be of especial value. As in previous techniques described for the preparation of bi-specific antibody molecules our procedure involves the fusion of two hybridomas, but in the present instance one of these produces antibodies directed against T-cells and the other produces antibodies directed against immunoglobulin. The two hybridomas may be fused by the conventional procedures, as illustrated in the Examples, to produce a hybridoma secreting bi-specific antibody molecules having each of the specificities of the hybridomas from which they derive. It will be appreciated, however, that one of the most difficult stages of any hybridoma-producing fusion is the selection from the fusion mixture of the desired type of bi-specific hybridoma and the procedure we have developed involves features directed particularly to this end.

In our procedure, the first hybridoma used has a drug selectable marker which may conveniently be an enzyme deficiency, for example of thymidine kinase (TK) or particularly of hypoxanthine-guanine phosphoribosyl transferase (HPRT). Such hybridomas are obtained by selecting cells from a growth medium containing 5-bromouracyl deoxyribose or 2-aminopurine (for TK) or 8-azaguanine or particularly 6-thioguanine (for HPRT). Cells selected for growth on a medium containing the appropriate drug lack the enzyme in question and, in the case of both TK and HPRT, will therefore be incapable of growth in a medium containing hypoxanthine, aminopterin and thymidine (HAT) since the aminopterin blocks the main pathway for purine and pyrimidine synthesis and the lack of HPRT or TK removes the ability possessed by normal cells of utilising the hypoxanthine to make purines and the thymidine to make pyridimines.

The second hybridoma employed in our procedure is poisoned with a lethal dose of an irreversible biochemical inhibitor, for example diethylpyrocarbonate and particularly iodoacetamide. Such an inhibitor poisons the cells but does not damage their DNA, which codes for immunoglobulin expression and also for th HPRT enzyme. Following the treatment of these cells they are washed to remove any excess of the inhibitor and are then ready for use, the overall structure of the cells remaining intact for several hours after the treat-

ment, the fusion typically being carried out within 0.5 to 1 hour.

Fusion of the two hybridomas will produce a fused cell system which possesses the DNA from both hybridomas and in which any short-term loss of vital enzyme function from the poisoned cells will be complemented by the enzymes derived from the other hybridoma. Following the fusion of the two hybridomas the fusion mixture is cultured in a medium free from inhibitors when cells of the unfused hybridoma which has been poisoned will gradually die whilst cells of the other unfused hybridoma and the fused cells will survive. Selection is then commenced with, for example, a HAT-containing medium when cells of the unfused hybridoma lacking TK or HPRT will die but the fused cells will survive since the enzyme deficiency is met by the DNA from the other hybridoma. Iodocetamide has been found to cause cell death within 1 to 24 hours but it has been found that a greater level of hybridisation is generally obtained if selection is delayed for 2 to 3 days, possibly because HPRT expression derived from the poisoned cell requires a little time to occur fully. Moreover, best results have been obtained using an equal or higher proportion of untreated cells to iodoacetamide-treated cells, for example from 1:1 to 10:1.

The mixture of cells from which both types of unfused hybridoma cell have been eliminated is then treated further in the usual way to isolate therefrom hybridoma secreting monoclonal antibodies having the desired bi-specificity. Such hybridomas may be cultured either *in vitro* or *in vivo* by conventional techniques in order to produce supplies of the monoclonal antibodies.

Although the use of the type of bi-specific antibody described herein of itself represents an improvement over the use of the bi-specific antibodies described in the art, in a preferred aspect of the present invention a further improvement may be obtained. Thus, it is quite possible for a bi-specific antibody to induce killing of the T-cells to which it binds through the natural Fc receptor mediated killing mechanisms referred to hereinbefore. Although no reference is made thereto in the Staerz and Bevan papers described hereinbefore, this is a serious deficiency in the effector cell retargeting procedure of the prior art since that procedure kills tumour cells only through T-cell toxicity. In the case of the present invention, the problem is less serious as other tumour killing mechanisms are also involved but it still reduc s the efficiency of tumour cell killing which is likely to be achieved. In a pref rred aspect of the present invention, therefore, the probl m of T-cell destruction may b mitigated, and pr ferably substantially avoided, in one of two ways.

The first of thes ways is based on an appreciation that the probl m can be overcome by th use of an appropriate combination of heavy chains in the bi-sp cific antibody molecules of the present invention. The overall structure of an immunoglobulin is determined by the interactions of the various globular domains of the individual chains with each other. These interactions consist of covalent bonds involving intra-chain and inter-chain disulphide bridges as well as non-covalent interactions involving both protein and carbohydrate groups. In order to activate the killing mechanisms, complement components and Fc receptors must bind to structures present in the different antibodies but different species, isotypes and allotypes of antibody have differences in portions of their protein sequences although they may have many similarities in other portions of these sequences. Thus different immunoglobulins will interact differently with complement components and Fc receptors and, in addition, when hybrid antibody molecules are made the two heavy chains may differ in sequence at crucial points for their interaction with each other and this may influence the properties of different combinations.

The present invention thus includes an antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, the two heavy chains in the molecule being selected so that an ability for the antibody molecule to utilise the natural killing mechanisms of the body to destroy the T-cells is mitigated.

The immunoglobulins which constitute antibodies may be divided into several classes, the major ones of these being identified as IgG, IgA, IgM, IgD and IgE, of which some, in particular IgA and especially IgG, may be further divided into subclasses. The bivalent antibody molecules according to the present invention preferably contain two heavy chains of the same class but may be of different subclasses within that class and may be of the same or a different subclass but relate to different species, the human, rat and mouse being of most interest, although immunoglobulins of other species, for example the rabbit, may be employed. Combinations of rat and mouse and rat and rat immunoglobulins are preferred to the mouse and mouse combination in terms of the higher level of stability of the corresponding hybridomas.

As regards the Fc receptor mediated killing mechanisms, particularly ADCC, we have found that although the nature of the heavy chain providing th T-cell binding affinity is of prime importance, the nature of the heavy chain providing the immunoglobulin binding affinity is also of importance. Thus, certain subclasses of immunoglobulin of a first species will not int ract with the cell m diated effector mechanisms of a second species and with thos subclasses which will interact when two

heavy chains of the same species and subclass are present in the immunoglobulin it is possible to interfere with this interaction by replacing one of the two heavy chains by an appropriate selection from another species or subclass. With the complement activation mechanism, the nature of the heavy chain providing the T-cell binding activity may be a more dominant factor in determining the level of complement activation which occurs than it is with ADCC, but the nature of the heavy chain providing the immunoglobulin binding activity can still exert an important effect as discussed hereinafter.

In selecting an appropriate combination of heavy chains for use in a bi-specific antibody molecule according to the present invention it must be appreciated that the species to which the two heavy chains relate and the species to which the antibody is to be administered are all of importance. Thus, a particular heavy chain species subclass combination which does not produce killing of T-cells in the mouse or rat, for example, may well do so in the human, which is the species of choice as regards the T-cell binding affinity of the bi-specific antibodies. As indicated hereinbefore, human heavy chains may conveniently be used for at least one of the heavy chains in the bi-specific antibody molecules of the present invention. Indeed, a preferred choice is the use of two human heavy chains of the same class but of a different subclass. However, the relative lack of availability of human myelomas as compared with mouse and rat myelomas can pose a problem and in practice these species may often therefore represent the mammalian species of choice for the heavy chains.

In the human, the IgG class is divided into the subclasses IgG1, IgG2, IgG3 and IgG4 (the IgA class being divided into the subclasses IgA1 and IgA2) whilst in the mouse and rat only the IgG class is divided into subclasses, these being IgG1, IgG2a, IgG2b and IgG3 in the mouse and IgG1, IgG2a, IgG2b and IgG2c in the rat (the similarly named subclasses not necessarily having similar properties in the mouse and the rat). In practice each of the heavy chains is most likely to be of mouse or rat IgA or IgF, particularly IgM and especially IgG, the commonest situation being that they are each of the IgG subclass and especially mouse IgG1, IgG2a or IgG2b, and less commonly IgG3, or rat IgG1, IgG2a or IgG2b, and less commonly IgG2c.

In selecting a heavy chain having T-cell binding activity, the simplest course is to use a class or subclass, for example, of the rat or mouse, which does not lead to the killing of T-cells in the species in question via an Fc receptor mediated mechanism. A convenient choice for the avoidance of ADCC killing, in particular, is a heavy chain of the IgM class but such heavy chains are often particularly effective at producing killing through complement activation. In terms of activity and of common availability a heavy chain of the IgG subclass is therefore an usual choice and for the avoidance of killing through complement activation the order of preference in the mouse is IgG1>IgG3>IgG2a=IgG2b and in the rat is IgG1>IgG2c>IgG2a>IgG2b. As regards the avoidance of killing through other Fc receptor mediated killing mechanisms, particularly via ADCC involving K-cells, the preference in the mouse is IgG2b, IgG3 and IgG1>IgG2a and in the rat IgG1 and especially IgG2a and IgG2c>IgG2b. We have discovered, however, that the class interaction between the two heavy chains in bi-specific antibody molecules, but not in conjugates, is such that even though the heavy chain of T-cell binding activity is one such as mouse IgG2a or rat IgG2b which will promote killing, it is possible to counteract this killing through the selection of a suitable form of heavy chain having immunoglobulin binding activity, in particular one of a different species or of a different isotype or allotype. Among the other rat IgG subclasses the preferences for inactivating the rat IgG2b heavy chain are a rat IgG2a or especially a rat IgG2c heavy chain. With such active heavy chains as rat IgG2b or mouse IgG2a combination with an alternative form of heavy chain, either by species (for example rat IgG2b/mouse IgG1) or by subclass, is indicated since a rat IgG2b/rat IgG2b or mouse IgG2a/mouse IgG2a combination can generally be presumed to be effective in promoting killing through the ADCC mechanism and possibly also through the complement activation mechanism. The use of heavy chains of a different allotype or particularly a different species or isotype can also be of value when the anti-T-cell heavy chain may exhibit only an insubstantial level of effectiveness in causing killing (i.e. being substantially ineffective), possibly acting at a low level through only one of these two mechanisms as may be the case for example with some mouse IgG isotypes. Although similar combinations of isotype may be used in such instances, the use of a difference of species or isotype will mitigate even the insubstantial level of effectiveness and provides a clear indication of suitability for the heavy chain combination. In general, the achievement of the mitigation of the killing of T-cells may be assessed by a comparison with such bi-specific antibody molecules in which the heavy chain having the affinity for target cells is the same (species, isotype and conveniently allotype) as that having the affinity for T-cells.

In addition to the use of the information given hereinbefore, simple test procedures may be used to determine appropriate combinations of heavy chains for use in the bi-specific antibodies of the present invention. Thus, if it is proposed to use in a bi-specific antibody molecule the heavy chain/light chain

combination f a particular monoclonal antibody against T-cells which is itself capable of inducing the killing of T-cells, then the hybridoma producing this monoclonal antibody can be fused with a hybridoma producing a specified immunoglobulin type of monoclonal antibody of any irrelevant binding affinity. The resulting bi-specific antibody can then be tested to see if the T-cell killing ability of the first heavy chain/light chain combination is negated by combination with a heavy chain/light chain combination of the immunoglobulin type in question. Thus, for example, by fusing a hybridoma producing a rat IgG2b antibody against T-cells with a hybridoma producing any irrelevant rat IgG2a or IgG2c antibody it can be tested whether the ability of the IgG2b heavy chain to induce killing by both the ADCC mechanism and through the complement activation mechanism is retained or not. An alternative approach is to transfect cloned immunoglobulin genes into a hybridoma so that the cloned gene is expressed in the hybrid cell and mixed immunoglobulin molecules produced which can be assayed for activity in T-cell killing by both the ADCC and complement route.

Although such procedures may be used with advantage to identify heavy chain combinations of particular value, it will be appreciated that in general any difference of species or difference of isotype (i.e. either of class or subclass) is sufficient to mitigate the killing of T-cells by the bi-specific molecule and that many differences of allotype will also achieve this result. Other criteria relating to the selection of combinations of heavy chains are discussed hereinafter.

Even though an appropriate combination of heavy chains not leading to the killing of T-cells is present in the bi-specific antibody, however, the procedures available for the preparation of that antibody, particularly when using hybridoma technology, can lead to a complex mixture of antibodies, some of which may show T-cell toxicity even though the bi-specific antibody does not. The various types of different antibody which may be present in the mixture obtained on preparing a bi-specific antibody molecule from two hybridomas not expressing a myeloma derived light chain are illustrated in the Fig. 1 which appears at the end of the specification. In theory, every possible combination of the two different light chains and two different heavy chains (shown in the Figure as black for those having T-cell binding activity and white for those having immunoglobulin binding activity) can occur and in practice some antibodies of each type may be presumed to be obtained although not necessarily in equal proportions. It is only in the cas wher th bi-specific antibody molecule heavy chain having a T-c ll binding affinity will not mediate the killing of T-cells by an Fc r c ptor mediated killing mechanism, such as ADCC or c mplement activation, that none of the types of antibody will be capable of destroying T-cells. In a case where this heavy chain will cause the killing of T-cells via an Fc receptor mediated killing mechanism, types 2 and 4 will be toxic via this route irrespective of the nature of the immunoglobulin-binding heavy chain, and types 1 and 5 will be toxic in addition to types 2 and 4 when the immunoglobulin-binding heavy chain is not such as to negate the activity of the other heavy chain. Accordingly even though the bi-specific type 1 monoclonal antibody molecules do not cause the killing of T-cells owing to the selection of an appropriate combination of heavy chains for the anti-T cell and anti-immunoglobulin halves of the molecule, types 2 and 4 will always possess the undesirable ability to kill T-cells.

It will be seen therefore that in the case of human therapy using bi-specific antibodies comprising different rat IgG combinations the order of value (for the anti T-cell/anti-immunoglobulin combination) is IgG2b/IgG2b<IgG2b/IgG2a (or 2c)<IgG2a (or 2c)/IgG2a (or 2c) or IgG2a (or 2c)/IgG2b since the first is toxic to T-cells as the bi-specific molecule, the second produces toxic molecules in admixture with the bi-specific molecule but the third and fourth do not produce molecules toxic to T-cells and the other types of molecule present in admixture act only as a diluent to the bi-specific molecules. Other rat IgG combinations of particular interest for human therapy are IgG2b/IgG1, which should not be toxic to T-cells as the bi-specific antibody but will provide toxic type 2 and 4 molecules, and especially IgG1/IgG2b which should not be toxic to T-cells as types 1, 2, 4 and 5. Alternatively, the four possible different combinations of IgG1 with IgG2a or IgG2c may be used, which would all be expected to behave generally similarly to the IgG2a (or 2c)/IgG2a (or 2c) combinations described above. As regards the mouse, the combinations of similar isotypes IgG1/IgG1, IgG2a/IgG2a and IgG2b/IgG2b are generally somewhat less preferred (particularly IgG2a/IgG2a for the reasons given for rat IgG2b/rat IgG2b above) than all of the possible dissimilar isotype combinations of IgG1, IgG2a and IgG2b, which are of interest. Some of these dissimilar isotype combinations will of course be of greater interest than others on similar reasoning to that applied in the rat case so that, for example, combinations in which the anti-T c ll binding is provided by mouse IgG2a will be expected disadvantageously to provide toxic type 2 and 4 mol - cules. The whole range of rat IgG/mouse IgG and mouse IgG/rat IgG combinations are also of some interest.

In a further aspect of the present invention, therefore, the mixture of antibodies containing the bi-specific antibody of the invention which

is produced by a hybridoma system derived from two fused hybridomas or two other fusion partners may be fractionated to enhance the proportion of the bi-specific antibody molecules therein and preferably substantially to separate the bi-specific antibody molecules from or at least to reduce the proportion of other species which are undesirable, i.e. any types of molecule which act as a diluent to the type 1 molecules and particularly any species which are toxic to T-cells by an Fc mediated receptor mechanism.

The techniques used for such purification are broadly analogous to those described in UK Patent Application 2,144,147A where the product it is desired to purify is one of type 4, being a monovalent monoclonal antibody. Such procedures include the use of affinity chromatography, ion exchange chromatogrpahy and chromatofocussing, particularly using fast protein liquid chromatography (FPLC—Pharmacia Trade Mark) and high pressure liquid chromatography (HPLC) techniques.

Affinity chromatography can be exploited using either an antigen-containing column (for example an immunoglobulin) which will select for those species of molecule having the correct combination of heavy and light chains for specificity or, alternatively, an anti-isotype or protein A column can be used to separate on the basis of isotype. Ion exchange chromatography relies on the fact that at different pHs the charge on a protein varies as different side chains ionize so that the binding of protein to a charged column can be affected by ionic strength. A powerful application of ion exchange chomatography involves the separation of fractions on a first column at a first pH followed by the use of a second column, at a second pH, the columns usually being of opposite charge so that cation exchange chromatography is followed by anion exchange chromatography, or vice versa. Chromatofocussing relies on the fact that at a particular selected pH, the protein has no net charge and will not bind to a charged column so that similar mixed proteins are separted on a basis of their pI. FPLC and HPLC offer different advantages and may be used in combination.

As indicated above, by an appropriate selection of anti-T-cell heavy chain which does not exhibit T-cell toxicity or by the combination with a toxic anti-T-cell heavy chain of an anti-immunoglobulin heavy chain of a different species or isotype it is generally possible to achieve the desired aim of the present invention to provide a bi-specific antibody molecule which substantially avoids the killing of T-cells. By the use of purification techniques as indicated above it is possible to remove other components toxic to T-cells which would otherwise cause a composition containing the bi-specific antibody molecules to exhibit T-cell toxicity.

It will be appreciated that the bi-specific antibody may generally b us d in the form of a fragment retaining the binding affinities of the whole molecule. More importantly, however, a second way of avoiding the destruction of T-cell by the bispecific antibodies of the present invention is to use the antibody in the form of a fragment of the whole antibody which retains the binding affinities but not any T-cell killing capacity, particularly a $F(ab')_2$ fragment. This approach depends on the fact that the $F(ab')_2$ portion of the antibody molecule is still capable of binding to T-cells through one of its heavy chain/light chain combinations but the lack of the Fc portion of the molecule means that the killing of T-cells by an Fc mediated receptor mechanism cannot occur. A further advantage of using $F(ab')_2$ portions is that, being smaller, they are more rapidly cleared by the kidneys.

The preparation of the $F(ab')_2$ portion of the antibody molecule requires the use of an appropriate enzyme system to effect cleavage at a suitable point in the heavy chains to thereby remove the Fc region of the heavy chains (or alternatively at least that of the anti-T cell heavy chain) whilst retaining the remaining portion of the two heavy chains, each linked to its light chain and also to the other heavy chain via disulphide bridges. It will be appreciated that different species and isotypes have different amino acid sequences in the cleavage area of the immunoglobulin and that an appropriate enzyme specific for the amino acid sequence in question must be used. Furthermore, where the two heavy chains differ in species or isotype not all cysteine groups in one chain will necessarily be connected through a bridge with a cysteine group in the other chain and the possibility of certain modes of cleavage yielding Fab rather than $F(ab')_2$ fragments must be considered. It will therefore be necessary to select an enzyme, for example pepsin, papain, V8 protease, etc., and conditions of pH, temperature and time for its use which are appropriate to the bi-specific antibody system in question in order to produce a $F(ab')_2$ fragment (or other fragment lacking only one Fc region) retaining the two specificities of the whole antibody (but not its ability to kill T-cells). Having selected the appropriate enzyme and conditions, as identified by the production of a product having the characteristics indicated above, the techniques for the production of the $F(ab')_2$ fragments of the bi-specific antibodies according to the present invention are broadly similar to those described in the literature for the preparation of the $F(ab')_2$ fragments of mono-specific antibodies.

The present invention thus includes (a) a $F(ab')_2$ fragment of a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, and also (b) a process for the preparation of

such a fragment which comprises treating the antibody molecule with a suitable enzyme system to effect cleavage thereof to yield this fragment.

It will be appreciated that the fractionation of the different antibody molecules of types 1 to 10 as discussed hereinbefore can still be of value where the $F(ab')_2$ fragment is used since, although the removal of other types toxic to T-cells is not necessary in this instance, all of the other types 2 to 10 dilute the proportion of the single active type 1 in the preparation, whilst types 2, 4 and 5 will compete for binding to T-cells and types 3, 6 and 7 for binding to immunoglobulin. The other types, particularly types 2 to 7, may therefore advantageously be reduced in proportion to type 1 or substantially removed. This fractionation may be carried out either before, or preferably after, formation of the $F(ab')_2$ fragment.

It will be appreciated that there has never been any suggestion that the bi-specific antibodies and fragments thereof which are the subject of the present invention might be used in a medical context. The present invention therefore further includes (a) a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, for use in surgery, therapy or diagnosis and also (b) the use of a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, for the manufacture of a medicament for use in the treatment of neoplastic or other disease.

The bi-specific antibodies and fragments thereof described herein may be formulated for use in various ways, which will however, usually involve the use of a physiologically acceptable diluent or carrier which will conveniently be sterile and preferably also pyrogen-free for certain uses. This may take various forms, for example phosphate buffered saline, saline, balanced salt solution and dextrose solution. However, phosphate buffered saline may be mentioned especially as often being suitable.

The composition may, if desired, be presented in unit dosage form, i.e. in the form of discrete portions containing a unit dose, or a multiple or sub-unit dose.

The anti-target cell antibodies described herein may also be utilised in fragment, for example $F(ab')_2$, form although the advantages in doing this are less than those described above for the bi-specific antibodies. Moreover, the anti-target cell antibodies, or fragments thereof, may conveniently be formulated in a broadly similar manner to the bi-specific antibody molecule, or fragment thereof. Indeed, it may be appropriate either to incorporate the two types of antibody into the same composition or into a kit, although the former may result in conjugate formation as described hereinafter. The present invention thus includes (a) a composition comprising a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, and an antibody molecule having a binding affinity for target cells, or a fragment thereof having such an affinity, and also (b) a kit comprising in association a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, and an antibody molecule having a binding affinity for target cells, or a fragment thereof having such an affinity. Further included by the present invention are products containing a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, and an antibody molecule having a binding affinity for target cells, or a fragment thereof having such an affinity, for simultaneous, separate or sequential use in the treatment of neoplastic or other disease.

Both types of antibody or fragment thereof may be administered in various ways, for example intravenously, intraperitoneally or possibly intracerebrally, the mode of administration being selected to be appropriate to the type and localisation of the tumour or other target cells and also for ease of administration by the clinician and for the safety of the patient. In general, however, parenteral administration, and particularly intravenous injection, will often be used. The two types of antibody may be employed simultaneously, separately or sequentially but it will usually be the case that the anti-target cell antibody will be administered either before or together with the bi-specific antibody and where the latter is the case, a suitable time delay will often be from 0.5 to 24 hours. As regards dosages of the two types of antibody, or fragment thereof, the exact dosages will depend upon the potency of the reagents, the tumour or other disease burden of the patient and the patient's body weight/surface area ratio. It may, however be stated by way of guidance that a dosage of between 1 to 25 mg of each antibody or fragment, for example approximately the same amount of each, will often be suitable, conveniently used in a 7 to 10 day regimen involving 1 dose of each per day, i.e. a 10 day course of treatment involving the administration of a total dosage of 10 to 250

mg of each antibody or fragment to the patient. It will be appreciated however that dosages outside this range may be used where appropriate although a particular advantage of the present invention is the low dosages which may be used in many cases, i.e. towards the lower end of the range stated or even below it, thereby possibly even avoiding the setting up of an immune response to the bi-specific antibody molecules and thus allowing repeated usage.

It will be appreciated that the present invention includes the use of a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, in conjunction with an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity, in the treatment of neoplastic or other disease. In particular, it includes a method for aiding the regression and palliation of neoplastic or other disease which comprises administering to a patient in need thereof amounts which together are therapeutically effective in achieving such regression and palliation of a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule, and an antibody molecule having an affinity for target cells, or a fragment thereof having such affinity. Alternatively, however, the bi-specific antibodies may be used in conjunction with the anti-tumour antibodies for the removal of neoplastic cells from bone marrow *in vitro*, thereby allowing autologous bone marrow transplantation to be used in the treatment of malignancy.

The use of the bi-specific antibody molecules of the present invention in indirect ECR offers many benefits over direct ECR as envisaged by Staerz and Bevan. Possible variations upon the simple use of the bi-specific antibody molecules together with the anti-target cell antibodies *in vivo* or *in vitro* as described hereinbefore include the removal of some peripheral blood mononuclear cells from the patient and the treatment of these cells briefly with anti-T-cell antibodies, such as the monoclonal antibodies YTH 12.5.14 or 12.5.14.2 described in Example 2, in order to activate the cells. A panel of anti-tumour monoclonal antibodies is then selected, for example against differentiation antigens, surface immunoglobulins, tumour associated antigens, etc., and appropriate antibodies or combinations of antibodies are used to target the tumour cells for lysis by activated effectors which are reintroduced into the body several hours later in the presence of the bi-specific antibody molecules, allowing excess anti-tumour cell antibodies to be eliminated. In addition to versatility, an important advantage of indirect ECR versus direct ECR is that both preactivated cytotoxic T-lymphocyte (CTL) and K-cell populations may be recruited to destroy a tumour target. If the Fc portion of a bi-specific antibody molecule with anti-T-cell and anti-target cell affinities is able to bind CD16 on activated K-cells or Fc receptors on other effector cell populations such as monocytes and neutrophils, and to bind CD3 on CTL, then the antibody will not be ideal for therapeutic use as it may induce reciprocal killing between CTLs and K-cells or monocytes, etc., similar to that observed between CTLs in the presence of bivalent CD3 Mab. Therefore the efficient use of direct ECR requires that the FcγRlow on K-cells or Fc receptors on effector cells must be either blocked, for example with a suitable CD16 monoclonal antibody, or the heavy chains of the bi-specific antibody molecule must be selected so that the Fc portion does not bind to FcγRlow or other Fc receptors. Alternatively, the Fc portion of an anti-T-cell/anti-target cell bi-specific antibody molecule may be rendered inactive by producing $F(ab')_2$ fragments. Unfortunately, these processes eliminate the potential for K-cell mediated ADCC against a target cell coated with the bi-specific antibody. It is advantageous to utilise both ECR and ADCC to focus killing against a target cell and K-cell killing can be exploited using indirect ECR because the anti-target cell antibodies can be administered separately from the bi-specific antibody molecules. In such a context it is preferred to select anti-tumour cell or other anti-target cell antibodies of the rat IgG2b isotype which have been shown to mediate potent K-cell killing by ADCC and the technology now exists to class-switch other isotypes of rat antibodies to the IgG2b isotype. On infusing treated effector cells back into the patient, the activated K-cells home to the tumour site and kill antibody-coated target cells via ADCC. Additionally, bi-specific antibody molecules selected for having a non-functional Fc portion mediate indirect ECR by focussing the CTL response against the same population of target cells.

It will be appreciated that the bi-specific antibody molecules according to the present invention potentially allow the administration of different antibody treatments at various time intervals. This permits clearance of an excess of a first anti-target cell antibody so that a second anti-target cell antibody can home efficiently via its antiglobulin specificity to the tumour cells bound by the remaining first antibody. This will reduce the formation of aggregates which can be removed very efficiently by cells of the reticuloendithelial system, etc. Additionally, with indirect ECR, the us firstly of an appropriate anti-target cell antibody will allow the activated K-cells to mediate ADCC against the target cell. This is followed by th

administrati n of bi-specific antibody mol - cules when the CTL population is also recruited to kill the same target cell via indirect ECR.

In addition to their use as described hereinbefore the bi-specific antibody molecules of the present invention may be used, either as the whole molecule or as a fragment thereof, in the preparation of bi-specific antibody conjugates having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for target cells. In this role the bi-specific antibody molecules of the present invention provide an intermediate capable of use to prepare a range of bi-specific antibody conjugates having the same T-cell binding affinity but differing tumour cell or other target cell binding affinities.

The present invention therefore includes a bi-specific antibody conjugate having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for target cells, characterised in that the conjugate comprises a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin linked to an antibody molecule having a binding affinity for target cells, or a fragment thereof retaining the binding affinities of the whole conjugate.

For this alternative use, the bi-specific antibody molecules of the present invention are combined *in vitro* with an antibody having a binding affinity for target cells such as is described herein, the conjugate then being administered *in vivo*. Although the conjugates could be prepared by chemical linkage it is of course preferable to use the anti-immunoglobulin affinity of the bi-specific antibody molecule for the anti-target cell antibody to provide the linkage. The conjugates may therefore conveniently be prepared by the simple admixture of solutions of a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin and an antibody having a binding affinity for target cells. It is preferred that the bi-specific antibody conjugates so prepared are not isolated but are administered directly to the patient. Conveniently, therefore, the preparation may be effected in a suitable medium for this purpose so that, for example, the two components may be contained in equal volumes of steril physiological saline. The mixture may then be left for a suitable period for reaction to b complete but not to allow any substantial degree of aggregation, such as for up to 1 hour, for example 30 minutes, and may then be administered, particularly parenterally.

All of the preferences expressed hereinbefore in relation to the separate bi-specific antibody molecules and anti-target cell antibodies and their use will of course continue to apply when these are components of the bi-specific antibody conjugates. Thus, for example, a $F(ab')_2$ or other fragment of the bi-specific antibody molecule may be combined with the anti-target cell antibody or a $F(ab')_2$ or other fragment thereof. Formulation for therapeutic use will be effected similarly and doses will be similar bearing in mind that the conjugates are a combination of the two separate components so that the dosages indicated for these separate component will be combined to provide the appropriate dosages for the conjugates.

The present invention is illustrated by the following Examples.

Example 1

Preparation of hybridoma producing monoclonal antibodies specific for the human CD3 antigen and for rat immunglobulin

(1) *Anti-CD3 component*

The Lou rat myeloma cell line Y3-Ag 1.2.3 (CNCM, I–078) is fused with spleen cells from a DA rat immunised with human lymphocytes according to the procedure described by Clark and Waldmann, Methods in Hematology, 1986, *13*, 1–20, and the fusion mixture worked up as described therein selecting for hybridomas producing monoclonal antibodies having specificity for the human CD3 antigen defined by reactivity with all human peripheral T-cells, cross inhibition with the mouse monoclonal antibodies such as UCHT-1 (Burnset *et al*, Journal of Immunology, 1982, *129*, 1451) and OKT-3 (US Patent 4361549), and imunoprecipitation. The hybridoma is hypoxanthine-guanine phosphoribosyl transferase positive ($HPRT^+$) and expresses on spleen cell-derived light chain and a second myeloma-derived light chain of the kappa-la allotype.

Selection is made from myeloma light chain loss variants by cell cloning on semi-solid agar (Clark and Waldmann, *ibid*) and then assaying for the loss of rat kappa-la allotype expression using a sensitive red cell haemagglutination assay (Clark, Methods in Enzymology, 1986, *121*, 548–556). The variant selected is cloned on semi-solid agar and then maintained in culture in Iscoves modification of Dulbecco's medium (IMDM-Gibco Europe) supplemented with 1 to 5% v/v foetal calf serum (FCS) and buffered with bicarbonate using 5% CO_2 in air.

For short term handling of the cells the bicarbonate is replaced by extra hepes buffer and NaCl to maintain the ionic strength.

(2) *Anti-rat immunoglobulin component*

The BALB/C myeloma cell line NSI/Ag 4.1 is fused with spleen cells from a SJL mouse strain immunised with pooled rat IgG in complete Freund's adjuvant (Springer *et al*, Hybridoma, 1982, *1*, 257–273).

The selected hybridoma produces a monoclonoal antibody having sp cificity for a particular rat allotype but this is not one corre-

sponding to the immunoglobulin produced by the hybridoma (1), thereby avoiding auto-reactivity. Selection is made for a variant of this hybridoma which is HPRT negative by effecting culture in increasing concentrations of a medium containing the selective drug 8-azaguanine (Clark and Waldmann, *ibid*). The selected HPRT$^-$ variant is cloned and cultured as for the hybridoma (1).*

* In a first variant of this procedure the hybridoma (1) is 8-azaguanine resistant and the hybridoma (2) is poisoned with iodocetamide in step (3) and in a second variant 6-thioguanine is used instead of 8-azaguanine in either the original or first variant procedures, and in a third variant the hybridoma (2) is a myeloma light chain loss variant as well as or instead of the hybridoma (1), or one or both hybridomas are derived from a myeloma which does not express a light chain.

(3) *Hybridoma-hybridoma cell fusion*

Prior to fusion the hybridoma (1) is treated in phosphate buffered saline (PBS) with 10 mM iodoacetamide for 30 minutes at 0°C. The HPRT$^-$ iodoacetamide-poisoned cells are washed with HEPES buffered IMDM to remove excess inhibitor and then mixed with the HPRT$^-$ hybridoma (2) cells in three different proportions:– (a) 3.5×10^7 cells hybridoma (1)+3.5×10^6 cells hybridoma (2) (10:1); (b) 3.5×10^7 cells hybridoma (1)+3.5×10^7 cells hybridoma (2) (1:1); and (c) 3.5×10^7 cells hybridoma (1)+3.5×10^8 cells hybridoma (2). In each case the cells are mixed in HEPES buffered IMDM and pelleted at $200 \times g$. Cell fusion is induced by treating the cell pellet for 2 minutes with 1 ml of a 50% w/v solution of polyethylene glycol 1500 in PBS whilst stirring (Clark and Waldmann, *ibid*). The cells are then washed once with HEPES buffered IMDM, resuspended in bicarbonate buffered IMDM containing 5% v/v foetal calf serum, plated out into 24×2 ml culture well plates at four concentrations from 8×10^5 to 2×10^5 and cultured at 37°C under 5% CO_2. On the following day a control containing iodoacetamide-treated but infused cells is typically totally non-viable, whilst in the cultures of the fused cells the majority of cells are observed to be viable.

After culture for 24 hours, selection is commenced with hypoxanthine, aminopterine and thymidine (HAT selective medium—Clark and Waldmann, *ibid*), the HPRT$^-$ hybridoma (2) cells being non-viable in this medium. Culture in the HAT selective medium is continued for 2 or more weeks and the following assays are then employed to screen for hybrid cell lines producing bi-specific antibody. Functional human CD3 antibody is detected using immunofluorescence to demonstrate binding on HPB-ALL (Adrian and Hutton, Journal of Clinical Investigation, 1983, *71*, 1649–1660), a human T-cell leukaemia cell line, such binding indicating the presence of both the heavy and the light chain of the hybridoma (1). Functional anti-rat immunoglobulin activity is detected using a rat antibody with anti-mouse Thy-1 antigen or other irrelevant specificity (which is of the appropriate allotype in relation to hybridoma (2)) fixed to the microtitre wells. Antibody capable of binding to such an antibody is detected with biotinylated YA 9/36.39 (Clark, Ph.D. thesis, University of Cambridge, 1982), a reagent specific for mouse IgG2, The plate binding assay is again employed to screen for antibodies containing mixed immunoglobulin chains derived from both parental cells, the biotinylated YA 9/36.69 being replaced by a biotinylated monoclonal antibody specific for the rat IgG sub-class of the hybridoma (1). Cells from wells which are strongly positive in all three assay procedures are cloned twice in semi-solid agar and a suitable clone is selected, this being maintained in culture as for the mono-specific hybridoma (1).*

* In a variant of this procedure the anti-rat Ig hybridoma (2) is replaced by a hybridoma specific for Ig of another species, for example the mouse or the human. In a further variant, which may conveniently be combined with the first, one or both of the hybridomas (1) and (2) are replaced by a hybridoma derived from a human myeloma, for example as described in European Patent Applications 0062409 and 0148644, the UK Patent 2086937 and in US Patent 4529694.

Example 2

Preparation of rat IgG2b anti-human CD3/mouse IgG2a anti-rat immunoglobulin bi-specific hybridoma LHC 49.18.2

The procedure of Example 1(3) was followed using as hybridoma (1) the hybridoma YTH 12.5.14 (this is identical to the sister clone YTH 12.5.22 described by Cobbold and Waldmann, Nature, 1984, *308*, 460–462) and as hybridoma 2) the hybridoma RG 11/15.5 (Springer *et al, ibid*). YTH 12.5.14 produces a rat IgG2b monoclonal antibody and expresses a myeloma-derived kappa light chain of the Ia allotype. The myeloma chain loss variant YTH 12.5.14.2 secretes a single light chain unreactive with all tested anti-rat kappa reagents and which may therefore be presumed to express a light chain of the lambda class. RG 11/15.5 produces a mouse IgG2a monoclonal antibody specific for the rat kappa 1b allotype light chain and expresses a kappa light chain.

The fusion mixture was screened using biotinylated NORIG 7.16.2 (Hale *et al*, Journal of Immunology, 1985, *134*, 3056), a reagent specific for rat IgG2b in the third assay procedure. The bispecific anti-human CD3/anti-rat kappa 1b allotype light chain hybridoma LHC 49.18.2 was selected following the cloning procedure.

Example 3

Preparation of mouse IgG2a anti-human

CD3/mouse IgG2a anti-rat immunoglobulin bi-specific hybridoma LHD 6.23

The procedure of Example 1 was followed again using as hybridoma (2) the hybridoma RG 11/15.5 referred to in Example 2 and this case using as hybridoma (1) the hybridoma OKT3.5.2 which is a reclone of the mouse IgG2a anti-human CD3 hybridoma OKT3 referred to in US Patent 4361549 and deposited with the ATCC, Rockville, USA, under the accession number CRI 8001. OKT3.5.2 has identical properties to OKT3, which is derived from the mouse myeloma P3-X63-Ag8U1, that secretes a mouse kappa light chain, through a fusion with spleen cells from a CAF1 mouse immunised with E rosette positive-purified human T-cells.

The fusion mixture was screened with an immunoglobulin isotype assay using a suitable rat IgG antibody with a kappa 1b allotype light chain fixed to the microtitre wells and biotinylated YA9/36.69 to detect RG11/15.5 specificity and complement mediated lysis of human T-cells to detect OKT3 specificity by indirect fluorescence. The bi-specific anti-human CD3/anti-rat kappa 1 b allotype light chain hybridoma LHD 6.23 was selected following the cloning procedure.

Example 4

Preparation of mouse IgG2a anti-human CD3/mouse IgG2a anti-rat immunoglobulin bi-specific hybridoma LHB 63.10

The procedure of Example 1 was followed using the hybridoma OKT3.5.2, which is described in Example 3, as the hybridoma (1) and as hybridoma (2) the hybridoma NORIG 7.16.2 which is an HL secreting hybridoma derived by a fusion between spleen cells from a BALB/c mouse immunized with rat anti-pertussis/pertussis immune complexes and the non-producing mouse myeloma NSO/u (NORIG 7.16.2 is a subclone of identical properties to the NORIG 7.16 described by Hale *et al, ibid*). NORIG 7.16.2 produces an anti-rat IgG2b mouse IgG2a monoclonoal antibody expressing a kappa light chain. The 8-azaguanine resistant HL clone used was NORIG 7.16.2 AG. Following fusion, the screening procedures involved complement mediated lysis of human T-cells to detect OKT3 specificity by indirect fluorescence and an ELISA assay using plates coated with an irrelevant rat IgG2b antibody. NORIG 7.1.62 specificity was detected using a specific biotinylated anti-mouse Ig reagent follow d by streptavidin-peroxidas (both from Amersham plc, England). Following the cloning procedur the bi-specific anti-human CD3/anti-rat IgG2b hybridoma LHB 63.10 was selected.

Example 5

Production of monoclonal antibodies from bi-specific hybridoma

Th bi-specific hybridoma produced in Example 1, 2, 3 or 4 is maintained in low serum culture (IMDM containing 1% v/v FCS) for 48 h urs at 37°C using 5% CO_2 in air. The culture supernatant is then concentrated by precipitation with ammonium sulphate added to a level of 50% w/v. The precipitate is redissolved in the minimum volume of water and is desalted into 20 mM tris buffer at pH 8.0 on Sephadex G25. The antibody is fractionated by hplc ion exchange chromatography on a TSK-5PW column (LKB Ltd.) Pooled fractions are desalted into PBS for assay.

Example 6

Tests of activity of rat IgG2b/mouse IgG2a bi-specific monoclonal antibodies in effector cell retargeting

(A)

(1) *Preparation of human cytotoxic effector cells*

Venous blood was collected from healthy donors and was defibrinated using glass beads. Mononuclear cells were isolated from the interface following density gradient centrifugation on Ficoll-Hypaque and were then washed into bicarbonate buffered IMDM containing 5% v/v FCS. These cells were divided into tissue culture bottles at 1×10^6 cells per ml in the same culture medium, the bottles having been pre-coated with anti-human CD3 antibodies, in particular those produced by the hybridoma YTH 12.5.14.2 of Example 2. After 3 days in culture at 37°C the effectors were subjected to a 7 day expansion in the same medium containing 10 U/ml rIL2 (Cetus).

The blast cells produced in this way also contain 10–15% Fc receptor positive cells (as detected by Fc rosetting) which are able to mediate ADCC. These ADCC effectors were inhibited by pre-incubating the once-washed effector cells in the same medium with a 1/600 dilution of the anti-Fc receptor antibody CLB-Fcr gran I (Tetteroo *et al*, Leucocyte Typing II, 1985, Volume 3, page 27—Springer Verlanger, New York) for 15 minutes at room temperature. This anti-CD16 antibody blocks K-cell mediated ADCC.

(2) *Effector cell retargeting assay*

The mouse Thy-1 positive cell line BW 5147 (CRL 1588, PHLS European Collection of Animal Cell Cultures, Porton Down, England) was used as the target cell line. Cells were maintained in exponential phase in IMDM containing 2% v/v FCS until required for assay. Approximately 5×10^6 were then spun down at 200×g and the pellet resuspended in 150 µl IMDM containing 300 µCi ^{51}Cr-sodium chromate. The cells were incubated at 37°C for 1 hour and were washed into HEPES buffered IMDM. The target cells wer then washed and divided into two tubes. The cells in the first tube were resuspended in 500 µl of HEPES buffered IMDM medium for analysis of direct ECR and th cells in the second tube w r resuspended in a similar volume of the sam

medium containing approximately 100 μg/ml of anti-Thy-1 monoclonal antibodies derived from the hybridoma YBM 29.2.1 (see Cobbold *et al*, ibid) for analysis of indirect ECR. After a 1 hour incubation at room temperature the cells in the second tube were washed once to remove excess YBM 29.2.1 and were resuspended in the same medium at a level of 2×10^5 viable cells/ml.

Two-fold dilutions of effector cells were set up in 50 μl volumes in U-bottom microtitrre wells and equal vol8 hybridoma, (abelled Thy-1 coated or uncoated target cells were added in a 50 μl volume of HEPES buffered IMDM medium to provide an effector:target cell ratio ranging from 32:1 to 0.5:1. Suitable dilutions of the antibodies to be tested for effector cell retargeting were added in a 100 μl of HEPES buffered IMDM to the wells and incubation was effected at 37°C for 4 hours, after which 100 μl of medium per well was collected for a determination of the released radioactivity by measurement of gamma radiation using a Phillips gamma counter, model PW 4800.

(3) *Determination of activity of LHC 49.18 antibodies and SHN 20.12 antibodies*

Supernatants were used (the culture being obtained as in Example 5) of (a) a culture of Y3-Ag 1.2.3 myeloma cells as a control, (b) a 1:1 v/v mixture from cultures of the parental hybridomas YTH 12.5.14.2 and RG 11/15.5 of the LHC 49.18 hybridoma, (c) a culture of LHC 49.18 hybridoma cells, and (d) a culture of SHN 20.12 hybridoma cells (SHN 20.12 is a rat IgG2b anti-human CD3/rat IgG2c anti-mouse Thy-1 bi-specific hybridoma prepared as described in Example 3 of a copending application of even date herewith claiming priority form UK Patent Application 8626412 in the names of Clark and Waldmann) and (e) a 1:1 v/v mixture from cultures of the parental hybridomas YBM 29.2.1 and YTH 12.5.14.2 of the SHN 20.12 hybridoma.

In a first set of experiments the assay procedure described under (2) above was used but with a constant effector:target cell ratio of 15:1 and with a dilution of supernatant (in 100 μl volume) ranging from 1/100 to 1/12800. The control Y3-Ag 1.2.3 cells and the parental mixture showed no significant activity as measured by the level of ^{51}Cr release with both unlabelled and labelled target cells (but see below in relation to parental mixture). As also expected, the SHN 20.12 antibodies showed a marked level of ^{51}Cr release for both types of target cell whilst the LHC 49.18 antibodies, which unlike the SHN 20.12 antibodies will cause only indirect ECR, gave a marked level of ^{51}Cr release with the labelled cells but only a similar level of ^{51}Cr release to the control and parental mixture for the unlabelled cells. In a second set of experiments the effector:target cell ratio was varied from 32:1 to 0.5:1 as described under (2) above in conjunction with supernatants at a single dilution of 1/100 in 100 μl volume. The results obtained are shown in Figs. 2a and 2b which relate to unlabelled and labelled target cells, respectively. The SHN 20.12 antibodies, which effect direct ECR, were found to be active with both uncoated and coated target cells (plot shown only in Fig. 2a for clarity of other plots in Fig. 2b) whilst the LHC 49.18 antibodies, which effect indirect ECR, are as expected effective only with coated target cells (plot shown only in Fig. 2b for clarity of other plots in Fig. 2a). However, the killing produced by indirect ECR with the LHC 49.18 antibodies as shown in Fig. 2b will be seen to be at least as effective as the killing produced by direct ECR with the SHN 20.12 antibodies as shown in Fig. 2a. (The slight activity observed for the parental mixtures is believed to be attributable to a small number of spontaneously arising microaggregates.)

(B)

The experiments described in (A) above were repeated using both the mouse lymphoma BW 5147 as a target and also the mouse Thy-1 positive lymphoma cell line EL-4 (A.T.C.C. reference number TIB 40) which was prepared for use in the same way as described in (A) for the BW 5147 cells. Each of the BW 5147 and EL-4 target cells were used in both unlabelled and labelled forms as described in (A) using for labelling monoclonal antibodies from the anti-Thy-1 rat IgG2c, k1b hybridoma YBM 29.2 as in (A) or alternatively, monoclonal antibodies from the anti-Thy-1 rat IgG2b, k1b hybridoma YTH 154.7.7 (Cobbold *et al*, Molecular Biology and Medicine, 1983, *1*, 285–304) or the anti-LC/7200 rat IgG2a, k1b hybridoma YBM 42.2 (Stenning *et al*, Molecular Biology and Medicine, 1983, *1*, 95–115). The labelled target cells were added to microtitre plates containing two-fold dilutions of effectors and a 1/100 dilution of supernatant was added (the cultures being obtained as in Example 5) of one of (a) a culture of Y3-Ag 1.2.3 myeloma cells as a control, (b) a 1:1 v/v mixture of cultures of the parental hybridomas, YTH 12.5.14.2 and RG11/15.5, of the LHC 49.18 hybridoma, and (c) a culture of the hybridoma LHC 49.18.

Figs. 3a and 3b show the results obtained with BW 5147 cells coated with YBM 29.2.1 and YBM 42.2 antibodies, respectively. Figs. 3c and 3d show the results obtained with EL-4 cells coated with YBM 29.2.1 and YBM 42.2 antibodies, respectively. The results obtained with both types of target cells coated with YTH 154.7 antibodies were similar to those obtained with YBM 29.2.1-coated target cells and are therefore not illustrated. Unlabelled target cells of both types were, as expected, not killed in the presence of any of the three types of supernatant.

It will be seen from Fig. 3 that the LHC

49.18 antibodies, but not the control or the mixture of parental antibodies, achieved a good level of killing of both BW 5147 and EL-4 via anti-target-cell antibodies binding to the two antigens Thy-1 and leucocyte common antigen.

CLAIMS

1. A bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, or a fragment thereof retaining the binding affinities of the whole molecule.

2. An antibody molecule or fragment thereof according to Claim 1, in which the receptor is a human T-cell receptor.

3. An antibody molecule or fragment thereof according to Claim 1 or 2, in which the second binding affinity is for an immunoglobulin of a different species to that species to which the T-cell receptor binding affinity relates.

4. An antibody molecule or fragment thereof according to Claim 3, in which the second binding affinity is for rat and/or mouse immunoglobulin.

5. An antibody molecule or fragment thereof according to any of the preceding claims, in which the second binding affinity is for immunoglobulin which does not correspond to the immunmoglobulin of either light chain or either heavy chain in the molecule.

6. An antibody molecule or fragment thereof according to any of Claims 1 to 5, in which the two heavy chains each separately are derived from the human, rat or mouse and are of the IgG class of immunoglobulins.

7. An antibody molecule or fragment thereof according to any of Claims 1 to 6, in which the two heavy chains in the molecule are selected to mitigate the killing of human T-cells by the molecule.

8. An antibody molecule or fragment thereof according to Claim 7, in which T-cell killing is mitigated by the use of a first heavy chain having a T-cell binding affinity which is substantially ineffective at promoting killing through Fc receptor mediated killing mechanisms.

9. An antibody molecule or fragment thereof according to Claim 8, in which said heavy chain is derived from the rat and is of the IgG2a, IgG2c or IgG1 sub-class.

10. An antibody molecule or fragment th r of according to Claim 9, in which the two heavy chains ar of th isotypes, for th anti-T-cell/anti-target c ll specificities respectively, of IgG2a/IgG2a, IgG2a/IgG2c, IgG2c/IgG2a, IgG2c/IgG2c, IgG2a/IgG2b or IgG2c/IgG2b.

11. An antibody molecule or fragment thereof according to Claim 8, in which said heavy chain is derived from the mouse and is of the IgG2b, IgG1 or IgG3 subclass.

12. An antibody m l cule or fragment thereof according to Claim 7, in which the first heavy chain having a T-cell binding affinity is effective at promoting killing through Fc receptor mediated killing mechanisms but T-cell killing is mitigated by the presence of a second heavy chain having target cell binding affinity which renders said first heavy chain less effective.

13. An antibody molecule or fragment thereof according to Claim 12, in which the first and second heavy chains are of a different species, isotype or allotype.

14. An antibody molecule or fragment thereof according to Claim 12, in which the first heavy chain is rat IgG2b or mouse IgG2a.

15. An antibody molecule according to Claim 13 or 14, in which the first and second heavy chains are of the same class.

16. An antibody molecule or fragment thereof according to Claim 15, in which the heavy chain is derived from the rat and is of the isotypes, for the first chain/second chain respectively, IgG2b/IgG2a or IgG2b/IgG2c or is derived from the mouse and is of the isotypes, for the first chain/second chain respectively, IgG2a/IgG1 or IgG2a/IgG2b.

17. A bi-specific antibody molecule having a first binding affinity for a human T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin characterised in that the two heavy chains in the molecule are of a different species or isotype, or a fragment thereof retaining the binding affinities of the whole molecule.

18. An antibody molecule or fragment thereof according to Claim 17, in which the two heavy chains are derived from different species among the human, mouse and rat.

19. An antibody molecule or fragment thereof according to Claim 17, in which the two heavy chains are either derived one from the rat and the other from the mouse or are of different rat or of different mouse isotypes.

20. An antibody molecule according to any of Claims 17 to 19, in which the first and second heavy chains are of the same class.

21. An antibody molecule or fragment thereof according to Claim 20, in which the heavy chains are both derived from the rat and are of the isotypes, for the anti-T-cell/anti-target cell specificities respectively, IgG2a/IgG2c, IgG2c/IgG2a, IgG2a/IgG2b or IgG2c/IgG2b or are both derived from the mouse and are of the isotypes, for the anti-T-cell/anti-target cell sp cificities respectively, of IgG1/IgG2b, IgG2b/IgG1, IgG1/IgG2a or IgG2b/Ig2a.

22. An antibody molecul or fragment thereof according to any of Claims 12 to 16, which is substantially free from antib dy molecules which hav two of said first heavy chains and in which at least on of these heavy chains is in combination with its corresponding light chain, and from fragments of

said antibody molecules retaining the binding affinities of the whole molecule.

23. An antibody molecule or fragment thereof according to any of Claims 17 to 21, which is substantially free from antibody molecules which have two heavy chains with said first binding affinity and in which at least one of these heavy chains is in combination with its corresponding light chain, and from fragments of said antibody molecules retaining the binding of the whole molecule.

24. A fragment of an antibody molecule according to any of the preceding claims which is either the $F(ab')_2$ fragment of the whole molecule or a fragment of the whole molecule lacking only that Fc region which is that of the heavy chain having a T-cell binding affinity.

25. A fragment of a bi-specific antibody molecule having a first binding affinity for a human T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin, said fragment being either the $F(ab')_2$ fragment of the whole molecule or a fragment of the whole molecule lacking only that Fc region which is that of the heavy chain having a T-cell binding affinity.

26. A fragment according to Claim 25 of an antibody molecule as defined in any of Claims 3 to 6.

27. A bi-specific antibody conjugate having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for target cells characterised in that the conjugate comprises a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin linked to an antibody molecule having a binding affinity for target cells, or a fragment thereof retaining the binding affinities of the whole conjugate.

28. An antibody conjugate or fragment thereof which contains a bi-specific antibody molecule or fragment thereof as defined in any of Claims 1 to 26 in combination with an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity.

29. An antibody conjugate according to Claim 27 or 28, in which the target cells are tumour cells.

30. A composition comprising an antibody molecule or conjugate or a fragment thereof according to any of Claims 1 to 29, together with a physiologically acceptable diluent or carrier.

31. A composition according to Claim 30, which additionally contains an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity.

32. A kit which comprises in association an antibody molecule or fragment thereof according to any of Claims 1 to 26, and an antibody molecule having a binding affinity for target cells, or a fragment thereof having such affinity.

33. A composition according to Claim 31 or a kit according to Claim 32, in which the target cells are tumour cells.

34. An antibody molecule or conjugate or a fragment thereof according to any of Claims 1 to 29, for use in surgery, therapy or diagnosis.

35. The use of an antibody molecule or conjugate or a fragment thereof according to any of Claims 1 to 29 for the manufacture of a medicament for use in the treatment of a neoplastic, viral or parasitic disease.

36. A method of aiding the regression and palliation of a neoplastic, viral or parasitic disease which comprises administering to a patient in need thereof amounts which together are therapeutically effective in achieving such regression and palliation of an antibody molecule or conjugate or a fragment thereof according to any of Claims 1 to 29 and an antibody molecule having a binding affinity for tumour, vira or parasite cells, or a fragment thereof having such an affinity.

37. A method according to Claim 36, in which the disease is a neoplastic diease.

38. A process for the preparation of a bi-specific antibody molecule or fragment thereof according to any of Claims 1 to 26, which comprises culturing a hybridoma which expresses said bi-specific antibody molecule in order to produce said molecule and thereafter, where appropriate, treating this to produce a fragment thereof.

39. A process for the preparation of a bi-specific antibody conjugate or fragment thereof according to Claim 27, 28 or 29, which comprises combining a bi-specific antibody molecule having a first binding affinity for a T-cell receptor capable of activating killing and a second binding affinity for immunoglobulin with an antibody molecule having a binding affinity for either or both of said target cells, or a fragment of either or both of said molecules, and thereafter, if appropriate, treating the conjugate to produce a fragment thereof.

40. A bi-specific antibody molecule or conjugate whenever produced according to the process of Claim 38 or 39, or an obvious equivalent thereof.

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